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Biological and Abiotic Degradation of Xenobiotic Compounds in Vitro Estaurine Water and Sediment/Water Systems

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16. ABSTRACT

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BIOLOGICAL AND ABIOTIC DEGRADATION OF XENOBIOTIC COMPOUNDS IN IN VITRO ESTAURINE WATER AND SEDIMENT/WATER SYSTEMS

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ABSTRACT

First-order biotic and abiotic degradation rate constants of 14 pesticides were determined in estuarine water and sediment/water slurry systems. Test systems used environmentally realistic concentrations of pesticides in sterile and nonsterile samples of water and sediment taken directly from the field. Thiobencarb, sulprofos, chlorothalonil, diclofop-methyl, fenthion, oxyfluorfen, methoxychlor, phorate, and trifluralin all showed significantly ($p <= 0.01$) more degradation in the presence of nonsterile sediment than in the presence of sterile sediment. Most of these nine pesticides biodegraded significantly faster in flasks containing sediment than in those with water alone. Endosulfan and PCNB, however, biodegraded faster in the absence of sediment. EPN and chlorpyrifos were degraded primarily by abiotic processes. Methomyl did not significantly degrade under any test conditions. Oxyfluorfen and chlorpyrifos were also slow to degrade, with half-lives of generally over two weeks in nonsterile sediment. Diclofop-methyl and phorate were the least persistent, with half-lives of a few days or less.

INTRODUCTION

The application of pesticides to agricultural areas adjacent to streams and rivers frequently can lead to contamination of these aquatic habitats. Surface runoff could move dissolved and sorbed pesticides into receiving estuaries where residence time is long and pesticide fate is controlled by processes such as photolysis, hydrolysis, complexation, oxidation or reduction, and biodegradation.

We have been interested in the contribution of biodegradation to the fate of pesticides in estuarine systems. Biodegradation is a poorly understood fate process and a difficult process to quantitate. Most pesticide biodegradation information is related to terrestrial environments and it is not clear how much of this information is applicable to estuarine areas. Continued emphasis on estimations of exposure concentration in aquatic ecosystems indicates the importance of building a data base on the biodegradation of pesticides in estuarine environments, particularly from the standpoint of biotic and abiotic degradation rates.

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Biodegradation rates of xenobiotic chemicals in aquatic environments have been assessed utilizing pure microbial cultures (29,32) natural assemblages of microorganisms studied in flasks (8,33,34,45) and microcosms (1,35,39). This paper examines the degradation of four herbicides, two fungicides, seven insecticides, and one acaricide in a shake-flask, biodegradability test (5). The test utilizes estuarine water and sediments with particular attention to realistic concentrations, use of unsupplemented water and sediment taken directly from the field, description of degradation rates relative to biotic and abiotic conditions, and variability in rate determinations.

MATERIALS AND METHODS

Chemical names and structures of the 14 pesticides investigated are listed in Table 1. Water and sediment samples from either Davis Bayou, MS, or Santa Rosa Sound, FL, were used in the degradation test. Davis Bayou is an estuarine tributary to the Mississippi Sound and, ultimately, the Gulf of Mexico. This area is characterized by a silty, clay sediment, a vegetative cover of Spartina alterniflora and Juncus roemerianus, and annual salinities ranging from 0 to 25 g/L. Santa Rosa Sound, within the Escambia River Estuary, opens directly into the Gulf of Mexico. Samples for this study were taken from a Range Point saltmarsh located on the sound. Bottom sediments at this site are typically sand covered with decaying detritus. Vegetation is predominantly Juncus roemerianus and, to a lesser extent, Spartina alterniflora. Salinity ranges from 15 to 30 g/L. Water samples were collected in a carboy and filtered to 3 μ before use in the tests. Sediments were collected by skimming the top 2 cm of the sediment bed with a scoop or Eckman dredge. Sand was removed by differential settling and the resulting sediment slurry sieved to 2 mm. Sediment concentration in the slurry was determined gravimetrically. Water and water-sediment slurry were stirred 24 h prior to the test.

A predetermined concentration of test chemical calculated to produce a final water concentration somewhat greater than the nominal 200 μg/L initial concentration was added to a 4- or 8-L glass bottle in acetone or hexane carrier. Solvent was removed by flushing with air or nitrogen, site water was added to the mixing vessel, and the solution was stirred. Periodically, samples were collected and analyzed for test compound. When the measured concentration of test chemical remained constant for at least two successive analyses, approximately 1 L of this amended water was transferred to each of four 2-L Erlenmeyer flasks representing the following treatments: nonsterile water, nonsterile sediment in water, sterile water, and sterile sediment in water. Duplicate or quadruplicate treatment flasks were prepared with some test compounds to check replicability of the procedure. All sediment flasks contained 500 mg sediment (based on dry weight) per L. Sterile flasks were amended with 20 mL of 37% (by weight) formaldehyde solution. Site water was added to each flask to bring total volume to 1 L and initial test compound concentration to approximately 200 μg/L. Method detection limits or solubility dictated starting concentrations for some compounds. Flasks were placed in a shaker incubator and incubated in the dark at 25°C and 140 to 150 rpm. The pH was measured at least every other day and maintained at ±0.2 units of that initially measured for site water by adjustments with 1N HCl or 1N NaOH. Duplicate samples were removed periodically from each flask and analyzed for

Table 1. Nomenclature, Action, and Structure of Test Materials.

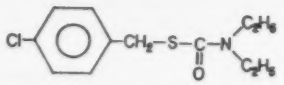
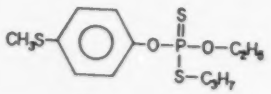
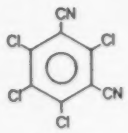
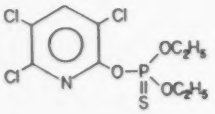
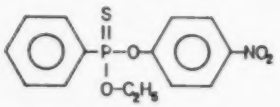
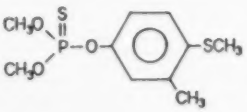
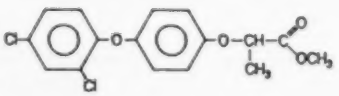
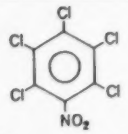
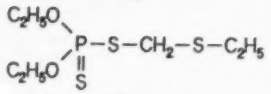
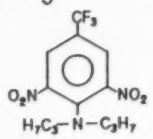
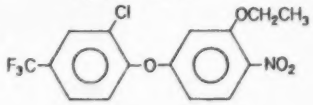
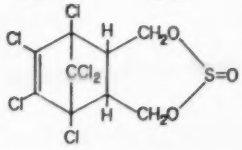
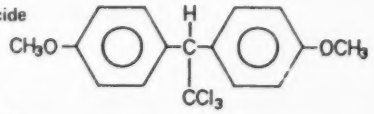
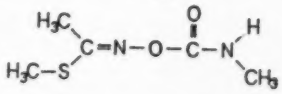
Common Name(s)	Chemical Name	Action	Structure
Thiobencarb	S-(4-chlorophenyl) methyl diethylcarbamothioate	Herbicide	
Sulprofos	O-Ethyl O-[4-(methylthio)phenyl] S-propyl phosphorodithioate	Insecticide	
Chlorothalonil	Tetrachloroisophthalonitrile	Fungicide	
Chloropyrifos	O,O-Diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate	Insecticide	
EPN	O-Ethyl O-(4-nitrophenyl) phenylphosphonothioate	Acaricide, Insecticide	
Fenthion	O,O-Dimethyl O-[3-methyl-4-(methylthio)phenyl] phosphorothioate	Insecticide	
Diclofop-methyl	Methyl 2-[4-(2', 4' -dichloro-phenoxy)-phenoxy] propanoate	Herbicide	

Table 1. (Continued)

Common Name(s)	Chemical Name	Action	Structure
PCNB	Pentachloronitrobenzene	Fungicide	
Phorate	O,O-Diethyl S-[(ethylthio)methyl] phosphorodithioate	Insecticide	
Trifluralin	a,a,a,-Trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine	Herbicide	
Oxyfluorfen	2-Chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl) benzene	Herbicide	
Endosulfan	6,7,8,9,10,10-Hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide	Insecticide	
Methoxychlor	2,2-bis(p-methoxyphenyl)-1,1,1-trichloroethane	Insecticide	
Methomyl	S-Methyl-N-[(methylcarbamoyl)oxy]-thioacetimidate	Insecticide	

residual test chemical. Control sediment and water systems (no test chemicals or formaldehyde added) were used to test for extraneous co-chromatographing peaks that might interfere with test chemical analysis and to determine extraction efficiency. Recoveries were > 85 percent for all chemicals.

Residues of all test chemicals except methomyl were determined by a single extraction of a 10-ml sample with pesticide grade hexane (1:1, v:v) followed by electron-capture gas-liquid chromatography (Walker et al., 1984; Pritchard et al., 1987; Cripe et al., 1987). Methomyl was analyzed by adding methanol to a 10-ml sample (1:10, v:v), filtering (0.5 μ), and quantifing with reverse phase HPLC using a 254 nm UV detector.

The first-order rate constant (k_1) for each chemical was determined by a SAS (Statistical Analysis System, SAS Institute, Cary, North Carolina) regression analysis (REG) using the model:

$$\ln C = a + k_1 t \quad (1)$$

where C is the concentration of the test compound (μg/L), a is the y-axis intercept, and t is time. Half-life ($T_{\frac{1}{2}}$) values were computed from the equation:

$$T_{\frac{1}{2}} = 0.693/k_1 \quad (2)$$

The F statistic was used to test the significance of the regression (compared to a slope of zero). A SAS general linear model (GLM) procedure was used to compare the rate constants of two or more tests. In some instances, an evaluation of the residuals indicated that another model (i.e., quadratic) might produce a better fit. We still felt this simpler linear regression model (Equation 1) was adequate for our needs since the r-squared values were generally high and first-order kinetics are used most frequently for modeling degradation processes. Duplicate samples from each flask were averaged prior to statistical analysis.

To determine significance of the fit of the data to the model and to compare degradation rates, we chose a p value of ≤0.01. Degradation rate constants from 22 sets of duplicate treatment flasks with seven compounds were not significantly different except in one instance when this p value was used. With this p value, there is little chance of a Type I error (null hypothesis = the rate is not significantly different from zero), where a rate is found to be significantly different from zero but actually is not, thus providing a greater measure of environmental protection. Finally, the occurrence of significant differences in rate constants between treatments (i.e., sterile and nonsterile), with only slight differences in half-lives, is infrequent at this p value; differences of a few hours or even days have minimal environmental significance.

RESULTS

Decreases in concentrations of test chemicals were observed in most flasks containing untreated samples of seawater and sediment slurry (Figs. 1 and 2). First-order decay rates in sterile and nonsterile flasks were compared in the presence and absence of sediment to determine if biotic factors were significant in controlling the observed decreases in parent compound concentrations. Nonsterile sediment and nonsterile water treatments were compared to examine the role of sediment in biodegradation. Rate constants are compared in Table 2; Figure 3 shows the range of half-lives for nonsterile water (A) and sediment (B) treatments for each pesticide.

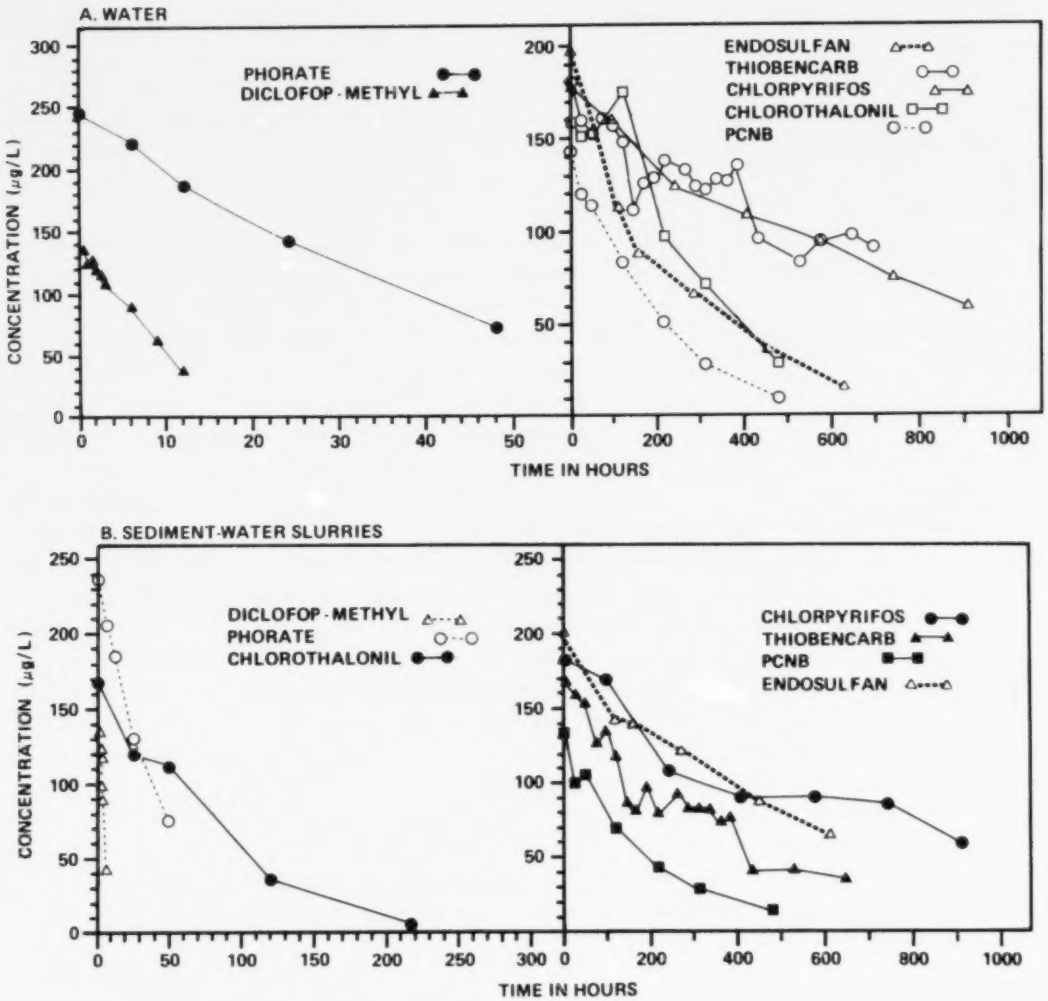


Figure 1. Persistence of pesticides in shake flasks containing water (A) or a sediment-water slurry (B) from Davis Bayou, MS.

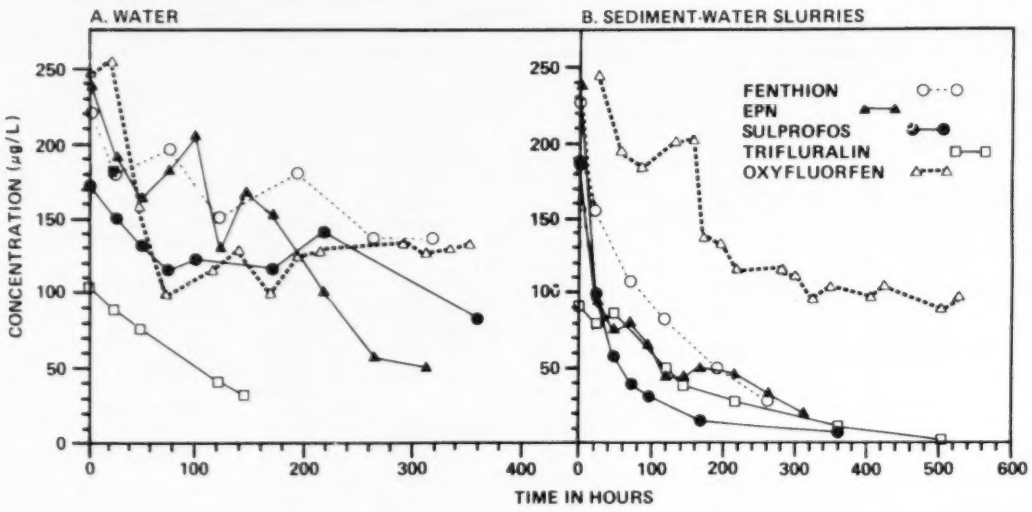


Figure 2. Persistence of pesticides in shake flasks containing water (A) or a sediment-water slurry (B) from Range Point, FL.

The presence of sediment in the flasks enhanced the biodegradation (rate constants from nonsterile sediment treatments were significantly greater than rate constants from sterile sediment treatments) of thiobencarb, sulprofos, chlorothalonil, diclofop-methyl, fenthion, oxyfluorfen, methoxychlor, phorate, and trifluralin. Degradation was primarily abiotic (sterile and nonsterile treatment rates were generally not significantly different) for chlorpyrifos and EPN. Abiotic degradation of endosulfan was affected by the presence of formalin; rates from sterile water and sterile sediment flasks were faster than rates from nonsterile counterparts. Degradation activity in nonsterile water activity was also significantly greater than activity from nonsterile sediment flasks for this compound. A flask study with autoclaved Range Point water produced an abiotic rate approximately 63% of that of formalin-sterilized water further confirming this formalin effect. For PCNB, activity in nonsterile water was significantly greater than that from sterile water or nonsterile sediment. Of the 14 pesticides, only methomyl showed no significant biotic or abiotic degradation. Oxyfluorfen and chlorpyrifos, with half-lives generally much greater than two weeks, were also persistent. Diclofop-methyl and phorate, with half-lives of a few days or less, were the least persistent pesticides.

Table 2. First-order rate constants for loss of 14 pesticides in biotic and abiotic shake-flask tests containing estuarine water or water and sediment.

Chemical	Site[a]	Date	Rep	First-Order Rate Constant (h^{-1})			
				Sediment		Water	
				Nonsterile	Sterile	Nonsterile	Sterile
Chlorothalonil	D	7/81	1	-0.0161[d,f]	-0.00574	-0.00355	-0.00283
			2	-0.0155		-0.00329	
Chlorpyrifos	D	3/80		-0.00165	-0.00177	-0.00179[f]	-0.00121
	R	2/81		-0.000945	-0.000562	-0.00109	-0.00144
	R	5/81		-0.00243	-0.00151	-0.00231	-0.00197
	D	5/81	1	-0.00111[f]	-0.000623	-0.00118	-0.00824
			2	-0.00102	-0.000908	-0.000703	-0.00130
			3	-0.00113		-0.00121	
			4	-0.00141		-0.00163	
Diclofop-methyl	D	8/80		-0.0883[d,f]	-0.0158[b]	-0.0457[f]	-0.00233[b]
	D	10/80	1	-0.225[d,f]	-0.0139	-0.103[f]	-0.00722
			2	-0.266	-0.0134	-0.120	-0.00785
Endosulfan	D	10/81	1	-0.00167	-0.00426[c]	-0.00374[e]	-0.0130[b,c]
			2	-0.00207	-0.00545	-0.00335	-0.00866
	D	11/81	1	-0.00165		-0.00490[e]	
			2	-0.00296		-0.00445	
	R	5/84		-0.00502	-0.00796[c]	-0.0157[e]	-0.0325[c]
EPN	R	6/79		-0.00586	-0.00315[b]	-0.00473	-0.00467
Fenthion	R	10/82		-0.00745[d,f]	-0.00199[b]	-0.00129[b]	--
Methomyl	R	8/82		-0.000215[b]	-0.000747[b]	-0.000175[b]	-0.000383[b]
Methoxychlor	R	5/84		-0.00236[d,f]	-0.000639[b]	-0.000139[b]	-0.00000327[b]
Oxyfluorfen	R	11/79		-0.00168[f]	-0.000315[b]	-0.00105	-0.000527
PCNB	D	7/81	1	-0.00456	-0.00380	-0.00554[e,f]	-0.00445
			2	-0.00481		-0.00633	
Phorate	D	8/80		-0.0241[f]	-0.0185	-0.0262[f]	-0.0185
	R	3/81		-0.0403[d,f]	-0.0209	-0.0206	-0.0186
Sulprofos	R	11/80		-0.00817[d,f]	-0.00152[b]	-0.00148[b]	-0.000469[b]
Thiobencarb	D	3/80		-0.00237[d,f]	-0.00337[b]	-0.000914[f]	-0.000349
	D	8/81	1	-0.000681[f]	-0.0000558[b]		
			2	-0.000688	-0.0000698[b]		
Trifluralin	R	1/81		-0.00504[f]	-0.00160	-0.0114[e,f]	-0.00499
	R	6/81		-0.00730	-0.00651	-0.00827	-0.00712
	D	8/81	1	-0.00621[d,f]	-0.00476	-0.00439[f]	-0.00299
			2	-0.0121	-0.00409	-0.00349	-0.00598

[a] D = Davis Bayou; R = Range Point.
[b] Rate constant not significantly ($p>.01$) different from a slope of zero.
[c] Sterile rate constant(s) significantly ($p\leq 0.01$) faster than nonsterile rate constant(s).
[d] Nonsterile sediment rate constant(s) significantly ($p\leq 0.01$) faster than nonsterile water rate constant(s).
[e] Nonsterile water rate constant(s) significantly ($p\leq 0.01$) faster than nonsterile sediment rate constant(s).
[f] Nonsterile rate constant(s) significantly ($p\leq 0.01$) faster than sterile rate constant(s).

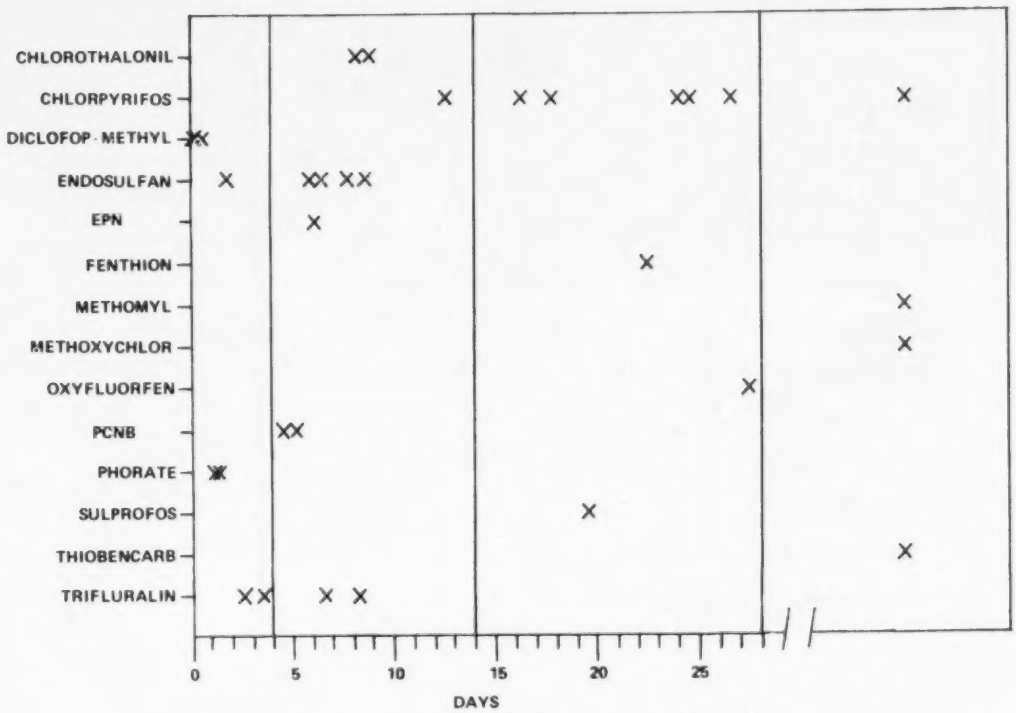


Figure 3A. Half-lives of 14 pesticides in non-sterile shake flasks containing water from Davis Bayou or Range Point (see Tables 1 and 2). Each point (x) represents an individual test flask.

DISCUSSION

We believe our method of assessing degradation by comparing rates of test chemical disappearance under specific test conditions is valid and definitive. Biodegradation of pesticides in water, for example, is assumed to occur if loss rates of parent compound in nonsterile water systems are significantly greater than loss rates in sterile water systems. No other mechanism readily explains the observed differences in rates between these systems. Factors, such as sorption to glass, volatility, photolysis, and pH, are common to sterile and nonsterile treatments.

Enhanced biodegradation of pesticides in the presence of sediments, determined here by significant differences in disappearance rates between sterile and active sediment flasks, occurred with 9 of the 14 pesticides studied. The effect of sediment on degradation has been reported for other chemicals (9,16,30,36,44,45). The mechanism responsible for this phenomenon

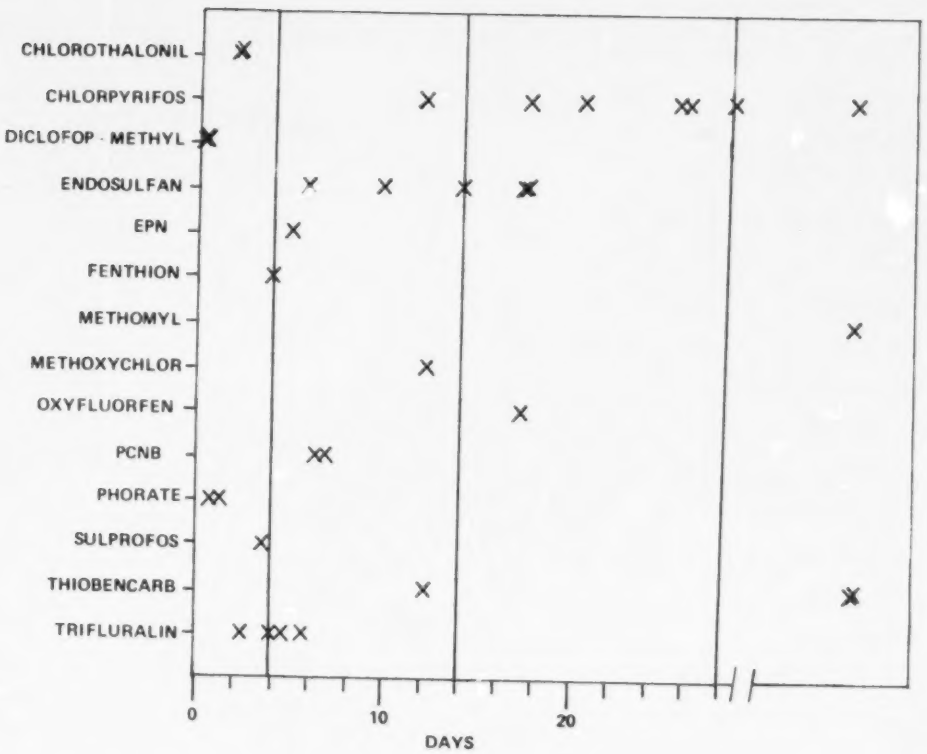


Figure 3 B. Half-lives of 14 pesticides in non-sterile shake flasks containing a sediment-water slurry from Davis Bayou or Range Point (see Tables 1 and 2). Each point represents an individual test flask.

is not clear. It could result from a greater number of degrader organisms on the surface of particles, an increased activity of degrading microorganisms in the presence of sediment (due to presence of nutrients or a surface for attachment), or an ability of sediment to concentrate the chemical, through sorption, making it more available to attached organisms (23). The magnitude of the rate increase over nonsterile water rates suggests that the fate of these pesticides and thus their exposure to nontarget organisms in estuarine environments will depend on contact of the chemical with sediment.

Disappearance in the presence of sediment, however, could be the result of irreversible sorption to sediment, relative to our extraction technique. However, any irreversible sorption should reach initial equilibrium (little further change in dissolved and sorbed concentrations) rapidly, and final equilibrium slowly thereafter (21). Most of our disappearance curves, in

contrast, showed a constant decrease in concentration with time. Irreversible binding of radiolabeled pesticides to soil has been observed in several studies (22,24). This binding occurs primarily under nonsterile conditions, suggesting that some microbial alteration of parent compound may be involved.

The presence of the chemical sterilant formalin could also produce misleading disappearance information. In this study, however, only one pesticide, endosulfan, disappeared faster as a result of the presence of formalin. We therefore believe that with the exception of endosulfan, the rate constants from sterile systems are a true reflection of abiotic degradation processes.

For purposes of comparing rates of degradation of the various pesticides, we have presented our results for nonsterile water (Fig. 3A) and nonsterile sediment (Fig. 3B) treatments in terms of half-life intervals of <4 days, 4 to 14 days, 14 to 28 days, and >28 days. Although the use of these intervals is arbitrary, there is precedent for assigning degree of risk intervals to chemicals based on their degradability (2,17,18). We feel that, based on persistence and assuming the absence of toxic degradation products, a chemical with a half-life of less than four days represents a minimum environmental hazard to the aquatic environment, while those with half-lifes greater than 28 days are of particular concern. The two intervals in between represent "gray" zones of concern. The ultimate risk posed by a compound will depend on integrating degradation test results with data obtained from toxicity, bioaccumulation, and other tests.

In 21 tests, duplicate flask treatments prepared simultaneously behaved as replicates (rate constants were not significantly different). Only chlorpyrifos disappearance in water from Davis Bayou on in May 1981 represented a problem with replicability of rate constants at our selected p value of 0.01. A pair of replicate sterile water treatments were significantly different ($p = 0.0009$), as were a set of quadruplicate nonsterile water treatments ($p = 0.0001$). Four replicate nonsterile sediment flasks run at the same time, however, did not have significantly different rate constants ($p = 0.1966$).

Quantitation of residual parent compound in our studies does not reveal the extent of degradation. Biotransformation or hydrolysis could result in the production of a product of similar or greater toxicity compared to that of the parent chemical. We have developed (5) an invertebrate bioassay test (Mysidopsis bahia for estuarine samples, Daphnia magna for freshwater samples) to assure that loss of acute toxicity coincides with disappearance of parent chemical.

Most environmental fate information on pesticides concerns soils. Results of shake-flask sediment degradation studies are generally comparable to aerobic, flooded soil treatments with a positive oxidation-reduction potential. Biodegradation of endosulfan in Davis Bayou and sediment treatments was similar to the half-lives obtained by Miles and Moy (26) for the α- and β-isomers in a soil culture inoculum (about 1.1 and 2.2 weeks, respectively). Biodegradation at the Range Point site was substantially faster than at Davis Bayou. It is not clear why biodegradation was slower in the presence of sediment than in its absence. Several studies (15,31) have suggested that some compounds are not available for biodegradation in the sorbed state, and therefore are more slowly degraded. However, at our sediment concentration (500 mg/L), it does not seem that enough chemical was sorbed to produce this effect.

Wakabayashi et al. (43) found EPN to persist in soil (half-life 30 to 90 days) but degrade relatively rapidly in submerged soil (half-life 3 to 15 days) or in the presence of Bacillus subtilis. These rapid decay rates were similar to those observed in our study. Wakabayashi et al. (43) indicated that biodegradation was important, since autoclaved soil degraded EPN much more slowly.

Freed et al. (10) indicated no difference in degradation between water samples with or without sediment, which was supported by our findings. Other investigators (19,38) have shown rapid disappearance of chlorpyrifos in water of ponds and artificial enclosures, but much of the loss may have been due to sorption on sediment and other surfaces of the enclosures rather than to biodegradation.

Wolfe et al. (47) studied the hydrolytic degradation of methoxychlor in distilled water and determined the half-life to be about one year at 27°C and typical aquatic pH values. This was comparable to the half-life in our nonsterile water flask, and supports our findings of a lack of significant abiotic or biotic degradation in the absence of sediment. Muir and Yarechewski (28) found the half-life of methoxychlor in air-purged flasks containing sediment and water (Eh +220 to 464 mV) to be 115 to 206 days, approximately one-tenth as fast as our nonsterile sediment treatment, and less than 28 days in sediments under nitrogen (Eh -50 mV).

Rapid disappearance of phorate from soil systems has been observed by several investigators (13,41,42). Chapman et al. (4) found that the phorate degraded much more slowly in autoclaved sediment than in nonsterile sediment, indicating the significance of biological degradation in the fate of this pesticide. We also observed rapid disappearance of phorate from all treatments. A statistically significant difference in rates observed between our sterile and nonsterile sediment treatments represented a difference in half-lives of less than one day; therefore, biological degradation is probably ecologically irrelevant in estuarine environments.

Trifluralin field application studies by Golab et al. (14) showed that about 14% of the original applied material persisted after one year. Similar field residues (10 to 15% remaining after 0.5 to 1 year) were reported by Probst et al. (37). In both cases, volatilization may have been the significant fate process. Willis et al. (46) found that redox potential significantly affected the degradation of trifluralin in soil suspensions: i.e., at +150 mV about 60% remained after 21 days, whereas at +50 mV almost all disappeared in 8 days. Our nonsterile sediment degradation rates were similar to those incubated at +50 mV.

The rapid, initial hydrolysis in soil of diclofop-methyl to its corresponding acid, diclofop, has been reported frequently (11,25,40). A very rapid degradation of diclofop-methyl also was noted in our studies, with half-lives of less than one day in nonsterile systems and half-lives of generally a few days in sterile systems. This compound degraded faster than any other pesticide we tested.

Thiobencarb degradation studies in soil (7) indicated that rapid breakdown was preceded by a 12-day lag period. Addition of compost or chemical fertilizers (N-P-K) reduced this lag; subsequent additions of the pesticide were rapidly degraded within 24 hours. Thiobencarb was shown to degrade much more slowly in flooded soils under anaerobic conditions (-270 mV) than

under aerobic conditions (+500 mV), whether in the presence or absence of added nutrients (7). Although our nonsterile sediment treatments showed some temporal variability in degradation, we observed rates comparable to those in the aerobic-flooded sediment conditions.

Approximately 37% of PCNB incubated aerobically with soil was extracted as the parent compound after 53 days (20). Chacko and Lockwood (3) found that eight actinomycetes and eight fungi degraded PCNB in pure culture, with a maximum degradation of 36% within about six days. Degradation in all of our shake flask treatments was comparable to this rapid disappearance rate. We noted, however, that PCNB degraded in nonsterile water at a significantly faster rate than in nonsterile sediment. Eichelberger and Lichtenberg (8) reported that 50% of the fenthion added to river water remained after 1 week. This is intermediate to our half-lives for nonsterile sediment and nonsterile water treatments and may reflect the presence of suspended particles in the river water they tested.

In our study, we determined degradation rates of 14 pesticides under biotic and abiotic conditions in the presence of estuarine water and sediment. Most compounds were significantly degraded by microbial activity without a lag period. The results underscore the important role that microorganisms associated with sediment play in the biodegradation process.

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